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Serum Transferrin Receptor as a Marker of Erythropoiesis Suppression in Patients on Chronic Transfusion

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In the management of patients requiring chronic transfusion, various parameters may be used to evaluate the degree of erythroid marrow suppression. The aim of our study was to assess which of these parameters provide the most useful assessment of erythropoiesis. We studied 27 chronically transfused patients, 19 with sickle cell disease (SS patients) and 8 with thalassemia. Thirty-one nonchronically transfused SS patients and 74 healthy children served as controls. We measured serum transferrin receptor levels, reticulocyte counts, hemoglobin (Hb) concentrations and erythropoietin levels. The serum transferrin receptor levels were very elevated in control SS patients and remained significantly elevated in those on transfusion therapy, but were normal in thalassemia patients, indicating a more complete suppression of erythropoiesis. The reticulocyte counts were elevated in all SS patients, even when on chronic transfusion, but were in the normal range in patients with thalassemia. Erythropoietin levels were elevated in patients with thalassemia and in all the SS patients. Hb levels negatively correlated with serum transferrin receptor and erythropoietin in all SS patients. In the transfused SS patients, a higher HbS level correlated with higher reticulocyte counts, transferrin receptor, and erythropoietin levels. In thalassemia patients, erythropoiesis was more completely suppressed, as reflected both by normal reticulocyte counts and near-normal transferrin receptor levels. Though the reticulocyte counts were not significantly different in the transfused SS patients, the serum transferrin receptor levels were less elevated than in SS patients not on transfusion. The serum transferrin receptor level appears to be the most useful marker of marrow erythropoietic activity in chronically transfused SS patients. We recommend that reticulocyte counts be integrated with periodic measurements of serum transferrin receptor levels. Am. J. Hematol. 60:121–125, 1999.

Key words: chronic transfusion; erythropoiesis; transferrin receptor; erythropoietin

INTRODUCTION

Membrane transferrin receptors play an important role in supplying transferrin bound iron to body tissues. The number of receptors determines the amount of iron uptake and varying numbers of receptors in different tissues account for the marked differences in uptake. In healthy humans, more than 80% of the body iron is ultimately used for erythropoiesis, and a proportionate number of transferrin receptors is found in the marrow erythroid precursors, by far the largest number being in the membrane of the normoblasts. As the latter mature through the reticulocyte stage, their receptors are gradually lost, along with their capacity to take up iron [1–3]. It has been suggested that serum transferrin receptor (TfR) measurements may provide a sensitive and rapid evaluation of erythropoietic activity in vivo, and that their levels may correlate closely with more complex and cumbersome erythrokinetic studies [4–8].

Nontransfused patients with sickle cell disease (SS pa-

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tients) and patients with thalassemia are expected to have an erythropoietin (EPO) level inversely proportional to their hemoglobin (Hb) level. In transfused thalassemia patients, erythropoietin levels have been analyzed in conjunction with serum TfR and Hb levels in order to find the best marker for erythropoietic suppression [9,10]. Singhal et al. and Serjeant et al. [11,12] observed in nontransfused SS patients that higher Hb levels corresponded to lower EPO levels. No such studies have been done in children with sickle cell disease on chronic transfusion therapy.

Chronic transfusion regimens are directed toward suppressing erythropoiesis. Patients with sickle cell disease are chronically transfused for a variety of reasons (primarily stroke or proven high risk for it, recurrent acute chest syndrome, etc.). The aim of this treatment is to keep the HbS at safe levels. The chosen level varies among institutions; at our center we aim at an HbS concentration below 20%.

Patients with severe homozygous β-thalassemia are transfusion-dependent for survival. A regular blood transfusion regimen is directed toward near complete suppression of the erythroid marrow, necessary to prevent bone deformities. In these patients, the goal is to keep the pretransfusion Hb >9–10 g/dL. At the time of diagnosis (in early infancy, prior to initiating the transfusion program), all had high reticulocyte and nucleated red cell counts, despite the ineffective erythropoiesis. However, TfR and EPO levels could not be measured at the time. It is thus not possible to compare these patients with a similar group not receiving transfusion.

We attempted to assess the residual erythropoietic activity in chronically transfused patients by evaluating Hb concentration, reticulocyte count, and serum TfR and EPO levels. In SS patients on chronic transfusion we also evaluated HbS percentage.

Our study was directed at assessing whether any of the studied parameters (reticulocyte count, serum TfR or EPO levels and Hb) provides a more useful assessment of erythropoiesis in chronically transfused patients and how well they correlate with each other.

MATERIAL AND METHODS

Plasma Samples

Serum samples from 32 SS patients not on chronic transfusion, 19 SS patients on chronic transfusion regimen, and 8 severe homozygous β-thalassemia patients followed in our clinic were analyzed. In chronically transfused patients, the samples had always been collected prior to transfusion. The average transfusion intervals for thalassemic patients were on the average 16 days and for SS patients were 23 days. From 74 normal healthy children, serum samples obtained from blood collected during their regular health maintenance visits for other reasons, were used in this study. All samples were stored at −20°C.

TABLE I. Average Hb, TfR, EPO, and Reticulocytes*

Group	Control	THAL	SS TX	SS
Number of Individuals	74	8	19	32
Age range (yrs)	2–15	11–20	3–20	1–19
Gender (M/F)	41/33	5/3	11/8	20/12
Number of measurements	74	70	136	32
TfR (pg/mL)	6.2 ± 0.2	9.1 ± 1.5	14.4 ± 1.4	31.6 ± 2.5
Reticulocytes (×1,000/mm^3)	n/a	33 ± 8.9	313 ± 28.9	384 ± 45.5
EPO (mU/mL)	7.1 ± 0.7	56.2 ± 13.1	47.0 ± 8.6	93.9 ± 8.6
Hb (g/dL)	11.8 ± 0.1	11.0 ± 0.2	9.9 ± 0.1	8.8 ± 0.3

*Hb, hemoglobin; TfR, transferrin receptor; EPO, erythropoietin; THAL, thalassemia; SS TX, sickle cell disease on chronic transfusion; SS, sickle cell disease. Mean ± standard error. (s.e. adjusted for correlation among repeated measurements.)

The patients and controls were divided into four groups: 1. Healthy controls; 2. Chronically transfused thalassemia patients; 3. SS patients on chronic transfusion; and 4. SS patients not on chronic transfusion. The age range, gender distribution, etc. in the analyzed groups are shown in Table I.

Multiple samples were analyzed from patients on chronic transfusions. Single samples were obtained from SS patients not on chronic transfusion and from normal children.

Serum Erythropoietin Assay

Circulating EPO levels were measured by a radioimmunoassay (EPORIA, Ramco Laboratories Inc., Houston, TX) which uses recombinant human erythropoietin for standards and tracer. Tests were run in duplicate.

Serum Transferrin Receptor Assay

The level of serum TfR was measured by an enzyme immunoassay (TfR, Ramco Laboratories Inc., Houston, TX). Tests were run in duplicate.

Hemoglobin, HbS, and Reticulocyte Measurements

Blood counts were obtained in the hospital hematology laboratory with a Coulter Counter STKS (Miami, FL). Reticulocytes were measured also in the hospital hematology laboratory with a Sysnex 3000 counter. The results were expressed as absolute number of reticulocytes/μL. Quantitative estimations of sickle hemoglobin (HbS) were obtained by electrophoresis.

Statistical Analysis

***t*-Test.** A modified *t*-test was used to compare the parameters of erythropoiesis in the different groups assuming intraclass correlation for repeated measurements in calculating the denominator of the *t*-test. This was needed to account for multiple measurements obtained in each of the patients undergoing chronic transfusion.

Correlations. Due to the lack of independence among repeated measurements within one patient, fitting a classical linear regression model would have yielded falsely understated standard errors. We therefore used a random effects model with intraclass correlation structure to take into account the correlation among repeated measures and to provide a valid standard error [13]. To examine the relation among the serum TfR, reticulocytes, EPO and Hb, we used Hb levels as dependent variable and EPO, TfR, and reticulocytes as covariates, one at a time. Within the two groups of transfused patients, we used TfR levels as dependent variables and EPO as a covariate. We also used the reticulocyte count as a dependent variable with TfR and EPO as covariates for each of these groups. Finally, using HbS concentration as a dependent variable and TfR and EPO as covariates, we analyzed their relation among the SS chronically transfused group.

RESULTS

Serum transferrin receptor. Compared with controls, TfR levels were slightly elevated in thalassemic patients (1.5 × control, $P = 0.01$), still elevated in the chronically transfused SS patients (>2 × control, $P = <0.01$), and most elevated in the control SS patients (>5 × control, $P = <0.01$). The difference between the latter two groups was significant ($P = <0.001$).

Reticulocyte counts. Reticulocyte counts were not significantly different from control in thalassemic patients ($P = 0.6$), were elevated in the transfused (>2 × control, $P = <0.001$) and control SS patients (>5 × control, $P = 0.001$), without significant difference ($P = 0.24$) between the latter two groups. In the control SS patients, reticulocyte counts positively correlated with TfR ($P = <0.01$). In chronically transfused SS patients there was no significant relation between reticulocyte count and EPO, and Hb correlated negatively with reticulocyte count ($P = <0.01$).

Serum erythropoietin levels. Serum erythropoietin levels were elevated in all groups compared with controls. They were markedly elevated in the thalassemia patients (>6 × control), most elevated in the control SS patients (>12 × control), and still high in the chronically transfused SS patients (>2 × control). There was a significant difference between the latter two groups ($P = <0.002$).

Correlation of hemoglobin with serum transferrin receptor and erythropoietin. The correlation showed no significance in thalassemia patients. However, the power of the test was low in view of the small number in this group ($n = 8$). The Hb levels were negatively correlated with both TfR and EPO in all SS patients (both on chronic transfusion or not; $P = <0.01$ for both). In chronically transfused SS patients, reticulocytes, EPO, and HbS positively correlated with TfR ($P = <0.01$). We had 68 measurements of HbS (mean = 16.7 ± 8.5%).

Levels of Hb, TfR, EPO, and reticulocyte counts in 3 SS patients prior to beginning transfusions were in the same range as the nontransfused group, and changed dramatically once the transfusions were begun. This number, however, is too small to perform statistical tests for significance.

DISCUSSION

The transferrin receptor is a transmembrane protein with 2 identical polypeptide chains, each weighing 95 kD. Iron delivery to red cell precursors and other rapidly dividing tissues is mediated by the interaction of serum transferrin with its receptors on the cell surface. The receptor binds serum diferric transferrin, is endocytosed, and transferred to acidosomes. Within these, inorganic iron is released into the cytosol and the remaining ligand and receptor are recycled back to the surface membrane. The soluble TfR present in human plasma is a truncated form of the tissue receptor and exists as a transferrin receptor complex [6].

Skikne et al. [14] performed serial phlebotomies in normal volunteers demonstrating an increase in serum TfR with progressive deficits in iron levels reflecting an increase in mass of receptors. The TfR expression on the cell surface reflects its iron requirement, and iron deprivation has been shown to result in the prompt induction of TfR synthesis.

Kohgo et al. [8] measured the serum TfR in patients with iron deficiency anemia, autoimmune hemolytic anemia, and aplastic anemia. They found elevations of TfR in iron deficiency anemia and autoimmune hemolytic anemia, and decreased values in aplastic anemia. These values paralleled the peripheral reticulocyte counts, suggesting that the serum TfR may reflect the turnover of transferrin receptors in the marrow erythrocyte progenitors, indirectly measuring erythropoietic activity.

A few previous studies analyzed serum TfR in thalassemia patients on chronic transfusion and its significance as a marker of erythropoietic suppression. In 1993, Musto et al. [10] compared EPO levels and serum TfR as markers of effective erythropoiesis suppression in severe β-thalassemia patients on chronic transfusion. They found the TfR to be a better indicator of erythroid marrow suppression in comparison with EPO or a fixed Hb

level. Their conclusion was subsequently confirmed by studies done in chronically transfused patients with thalassemia by Cazzola et al. in 1995 [9] and Al-Homaidi et al. in 1996 [15].

In SS patients not on chronic transfusions, studies of serum TfR were reported from the West Indies [11,12]. A negative correlation was observed between Hb levels, and serum TfR, as well as a partial correlation of EPO with TfR, as confirmed in our study.

SS patients have a very active erythroid marrow and thus a greater iron turnover. SS patients not on chronic transfusion had a markedly elevated serum TfR and EPO, which correlated well with each other as well as with the Hb level and reticulocyte count.

The goal of chronic transfusion in SS patients is to suppress red cell production enough to maintain a low HbS concentration. Thus, erythropoiesis suppression need not be complete. In chronically transfused SS patients, the average TfR level was significantly lower as compared with SS patients not chronically transfused ($P = <0.0001$), but it still remained considerably higher than in normal subjects ($P = <0.001$). These findings indicate that in SS patients, even on chronic transfusion, erythroid marrow remains still more active than normal.

In patients with severe homozygous β-thalassemia, the aim of chronic blood transfusions is to completely suppress erythroid marrow activity to prevent bone deformities. Therefore, Hb levels are maintained at higher levels with the aim of reducing erythroid marrow stimulation. In these patients, while the reticulocyte counts were constantly low supporting the more complete erythroid marrow suppression, the serum TfR was not significantly higher than normal. Thus, the serum TfR was significantly lower in thalassemic patients than in transfused SS patients, as the degree of marrow suppression was more complete in the former group. In thalassemia patients the EPO level remained elevated, almost in the same range as in chronically transfused SS patients. In thalassemic patients ferrokinetic measurements have shown that erythropoiesis, despite a significant decrease, still remained two to three times basal at an Hb level of 11 to 12 g/dL [9]. Since in both groups of patients the Hb levels remain below normal, this could trigger EPO secretion. The EPO levels probably represent the message sent to increase erythropoiesis, while TfR probably reflects the actual erythropoietic activity. Our study was directed to assess which parameters provide the most useful assessment of residual erythropoiesis. Our findings suggest the following conclusions:

1. In thalassemia patients, both the reticulocyte count and the TfR were in the normal range, indicating a nearly complete suppression of excess erythropoiesis.
2. In SS patients on chronic transfusion, despite higher Hb, the reticulocyte counts were not significantly de-

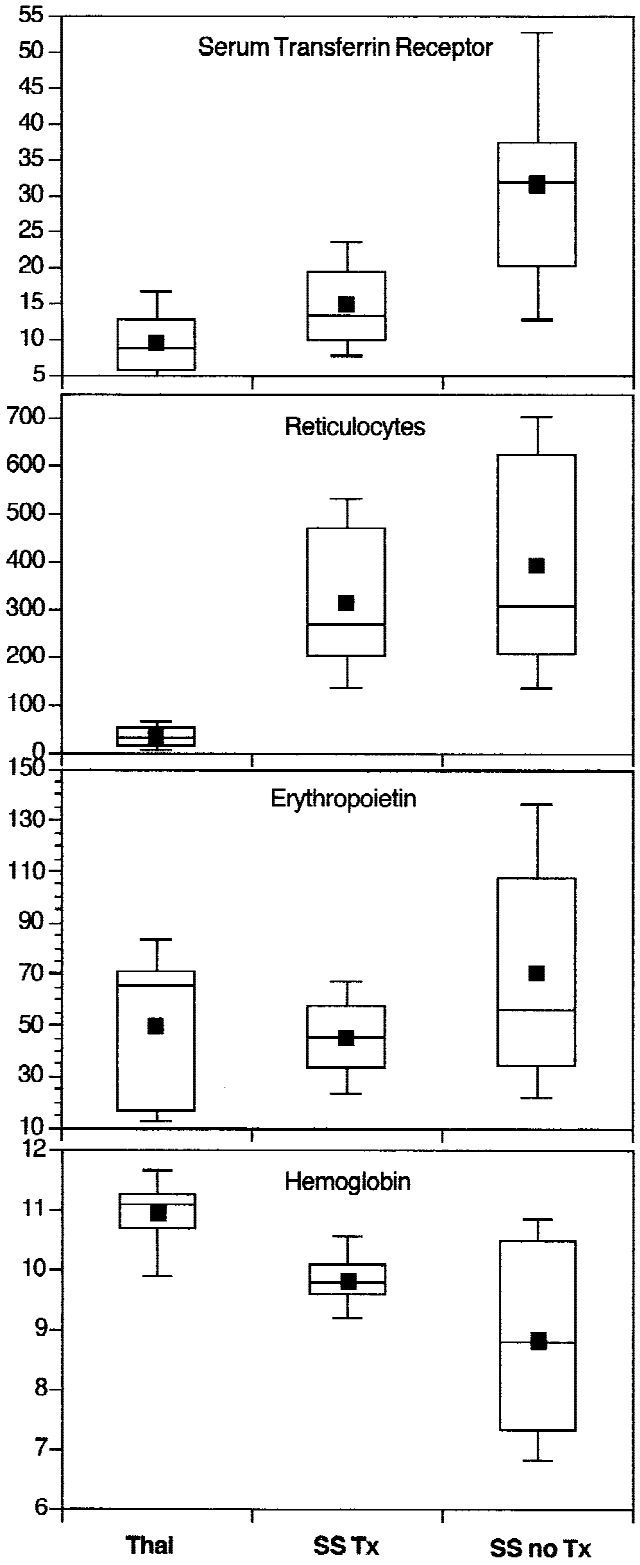

Fig. 1. Range of values for TfR, reticulocytes, erythropoietin, and Hb levels for thalassemia patients, SS patients on chronic transfusion, and SS patients not on chronic transfusion. In each group, the dark square indicates the mean, the center line indicates the median, the upper and lower lines represent the 75th and 25th percentage, and the caps indicate the range of values. TfR, pg/mL; reticulocytes, ×1,000/mm³; erythropoietin, mU/mL; Hb, g/dL.

creased in comparison with those not on transfusion ($P = 0.24$). The serum TfR was significantly lower than in SS patients not on chronic transfusion. It remained elevated indicating, as did the reticulocyte count, a residual active erythropoiesis.

3. Both in SS patients on chronic transfusion and in patients with thalassemia, the erythropoietin levels remained elevated, suggesting a signal to stimulate erythropoiesis, since in both groups the Hb levels remained subnormal.
4. Reticulocyte counts and serum TfR are correlated with each other and both appear to reflect erythropoietic activity in chronically transfused patients. In SS patients on chronic transfusion, the reticulocyte count was not significantly different from the level in nontransfused patients, but the TfR level was lower than in nontransfused patients. In patients with thalassemia, the reticulocyte count was in the normal range too low to be measured with precision, while the serum transferrin receptor can be more easily measured. Thus, in chronically transfused SS patients, and probably in thalassemic patients as well, the serum transferrin receptor appears to be the better indicator of suppression of erythropoiesis.
5. We suggest, therefore, that in patients on chronic transfusion, reticulocyte counts be obtained routinely and be integrated by periodic assessment of the transferrin receptor levels, to optimally evaluate the degree of residual endogenous erythropoiesis.

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